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Characteristics and Toxicological Processing of Postmortem Pilot Specimens From Fatal Civil Aviation Accidents

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16. Abstract

Autopsied biological samples from civil aviation accident pilot fatalities are submitted to the Civil Aerospace Medical Institute (CAMI) for toxicological evaluation. However, such evaluation is dependent upon types and amounts of submitted samples, and obtaining suitable samples is governed by the nature of an accident. Characteristics of those samples and associated toxicological processing have not been well documented in the literature. Therefore, the CAMI Toxicology Database was searched for these aspects. CAMI received samples from the pilot fatalities (CAMI cases) of approximately 80% of the 1990-2000 aviation accidents reported by the National Transportation Safety Board. Accidents and cases during June-September were higher than the other months, and more than half of the received cases had multiple samples in sufficient amounts. For example, out of 1,891 cases processed for the 1996-2000 accidents, 1,211 had at least adequate amounts of blood, urine, and/or vitreous humor; 324 had inadequate amounts of blood and urine; and 356 had no blood or urine. Muscle, liver, lung, and/or kidney samples were submitted in 90% of the cases, while cerebrospinal fluids were submitted in only 8% of the cases. The toxicologically preferred samples, blood and urine, were available in 78% and 56% of the 1,891 cases, respectively. Out of 51 cases containing only one sample type, 46 had muscle and the remaining 5 had other sample types. Samples were primarily analyzed for combustion gases, alcohol/volatiles, and drugs. Generally, the presence of analytes is demonstrated in at least 2 different sample types by using 2 different analytical techniques for reporting a particular case as "positive." An effective quality-assurance/quality-control is maintained throughout the process. In the majority of the aviation accidents, sufficient amounts and types of biological samples were submitted for toxicological evaluation.

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Characteristics and Toxicological Processing of Postmortem Pilot Specimens[1] From Fatal Civil Aviation Accidents

INTRODUCTION

For the investigation of aircraft accidents occurring within the jurisdiction of the United States, autopsied biological samples collected from the victims of fatal civil aircraft (air carrier and general aviation) accidents are submitted to the Federal Aviation Administration's (FAA's) Civil Aerospace Medical Institute (CAMI) for toxicological evaluation (2). Such sample submission is coordinated through the FAA's Office of Accident Investigation by the National Transportation Safety Board (NTSB), which is responsible for investigating all United States civilian aircraft accidents. In the majority of cases, the submitted postmortem samples are from pilots and copilots. However, depending upon the nature of an accident—for example, an accident involving fire—samples from passengers and other crewmembers are sometimes submitted.

The submitted biological samples are routinely analyzed for the presence of combustion gases, alcohol/volatiles, and drugs (7,8,10,16). Most of the drugs present in the samples from pilots/copilots are in the subtherapeutic to therapeutic concentration range (7,8,16), which is consistent with the nature of the postmortem aviation toxicology and could basically be referred to as the human-performance associated postmortem forensic toxicology (13). Therefore, multiple types of postmortem specimens in sufficient amounts are needed for the toxicological analysis; however, this requirement frequently cannot be achieved in accidents wherein victims' bodies are scattered, disintegrated, commingled, contaminated, and/or putrefied.

Limited information is available on characteristics and toxicological processing of postmortem samples from aviation pilot/copilot fatalities (cases) and associated accidents. Therefore, the CAMI Toxicology and the FAA National Aviation Safety Data Analysis Center (NASDAC) databases were searched to obtain the applicable information.

MATERIALS AND METHODS

Databases

Since 1990, a toxicology database for civil aircraft accident fatalities has been maintained at CAMI (Oklahoma City, OK). This CAMI Toxicology Database was searched to ascertain the number of aviation accidents and associated fatalities (cases), occurring between 1990 and 2000, from which CAMI received postmortem biological samples. In this paper, such accidents are referred to as "CAMI accidents" and related fatalities as "CAMI cases." These fatalities primarily consisted of pilots and copilots. Therefore, the "pilot fatalities" phrase used in this paper means pilot, as well as copilot, fatalities. Because of early database limitations, the data pertaining to the types and amounts of received-samples and other related information could not be completely retrieved for the accidents occurring prior to 1996. However, this information was effectively obtained from the database for the period of 1996-2000 and is accordingly incorporated in this research report. The number of fatal aviation accidents reported by the NTSB for the 1990-2000 period was obtained from the FAA's NASDAC Database, Washington, D.C.

Postmortem Aviation Forensic Toxicology Samples

Biological specimens collected during autopsy are packed with refrigerant packs in the styrofoam box of the FAA's TOX-BOX evidence containers. These containers with specimens are subsequently shipped to CAMI by an air carrier service for next-day delivery. It is strongly recommended to the TOX-BOX users that they use a single TOX-BOX evidence container for shipping samples from a particular fatality. Shipping samples from another fatality requires a separate TOX-BOX evidence container.

The supply of newly assembled TOX-BOX evidence containers with kits is maintained at various FAA Flight Standard District Offices. These containers with kits are regularly supplied by CAMI in advance

[1] The synonym "sample" is interchangeably used for "specimen" throughout the report.

to those offices, so that those boxes/kits are readily available in an event of an accident. If additional boxes/kits are needed at a particular site, CAMI ships the requested number of boxes/kits by an air carrier service for next-day delivery.

Details of the contents of the TOX-BOX kit are given in Table I. Gray-top and green-top glass tubes are for blood samples; red-top glass tubes are for vitreous humor, spinal fluid, and/or bile samples. Plastic bottles are for urine, gastric content, and/or bile samples; plastic bags are for solid tissue samples. Recommended quantities for different types of biological samples required by CAMI for toxicological evaluation are provided in the kit instruction sheet (Table II).

It is assumed that the external specimen chain-of-custody (ESCOC) and accident information forms are properly completed following the instructions provided with the kit, and all sample containers are properly sealed and labeled. Necessary information—such as name of victim, date and time of sample collection, type and amount of sample, and initials of sample collector—must be legibly written on the labels of the respective sample containers. The names and addresses of the coroner or medical examiner and of the accident investigator should also be included on the forms.

Sample Accessioning

Fatality samples are received in the accessioning room of CAMI's Toxicology and Accident Research Laboratory. Only authorized individuals are allowed in this restricted area. Individuals involved in performing analyses do not have access to the area. Unauthorized individuals are always escorted by an authorized employee.

Each fatality is given a unique CAMI case number, and an internal chain-of-custody (ICOC) form is prepared for the case. This process entails obtaining and incorporating information for the pilot/copilot and for the associated accident from the completed ESCOC and accident information forms, the FAA Administrator's Daily Alert Bulletin, the NTSB Website, the airman and medical certification records, and other available authentic and reliable sources. Examples of the ICOC preparation items are: name, birth date, airman and medical certificate numbers, and social security number of the pilot/copilot; date, place, and manner of the accident; aircraft tail number; and NTSB reference number. The ICOC form also includes a complete cataloging of the samples received, which encompasses sample types and any characteristic appearances (bloody, burned, cherry red color, coagulated, clotted, green, putrefied, etc.), sample container types and their weights with samples; markings and notes on containers; and any other relevant information. Known medical conditions of the pilots/copilots and/or any reported medications are noted on the ICOC form.

Analytical Batch Preparation

Depending upon the types and amounts of samples received, carboxyhemoglobin (COHb), blood cyanide (CN^-), alcohol/volatile, glucose, and hemoglobin A_{1c} (HbA_{1c}) analysis and drug screening batches are prepared and submitted for analysis. Batches are prepared by transferring a portion (aliquot) of the specimens of a case into test tubes. Generally, batches are comprised of samples from more than one case. Each batch also contains aliquots from at least 2 quality-assurance/quality-control (QA/QC) blind samples—1 negative QA/QC blind sample and 1 positive QA/QC blind sample. Only on rare occasions, 2 negative QA/QC blind sample aliquots are used. This generally happens when a positive QA/QC blind sample for a particular analyte is not available, primarily because of the newness, uniqueness, and/or rareness of the analyte. Amounts of aliquots used for a particular batch depend upon the type of the batch. The location of the blind sample aliquots is randomly changed from batch to batch. Similarly, where applicable and possible, concentrations and types of analytes in the QA/QC blind samples are also changed. The QA/QC blind sample aliquots are consistent with batch types, case aliquot matrices, and analytes.

Analysts do not know the location of and the type or amount of analyte(s) present in the QA/QC blind aliquots of a particular batch. Batch aliquots are analyzed by using standard operating procedures and practices established for the laboratory. After the completion of analyses, the documentation for the batches with the analytical data is submitted by the analysts for independent review.

If the initial analysis/screening is positive, additional samples are analyzed for confirmation or quantitation of the analyte(s). For such analyses, new confirmatory/quantitative batches are prepared with necessary QA/QC blind sample aliquots and are submitted for analysis. Most of the time, an aliquot from the sample type in which the analyte was detected during the initial analysis/screening is included in the confirmatory/quantitative batches. These batches also contain an aliquot from another sample type of the case, provided that is available. Specimens of a par-

Table I. Contents of CAMI's Tox-Box Kit

Description of Contents
One instruction sheet
One external specimen chain-of-custody (ESCOC) form with completed example
One accident information form with completed example
Three 10-mL gray-top glass tubes* (Vacutainers) in a styrofoam holder/protector with identification (ID) labels affixed
Three 10-mL green-top glass tubes† (Vacutainers) in a styrofoam holder/protector with ID labels affixed
Three 10-mL red-top glass tubes‡ (Vacutainers) in a styrofoam holder/protector with ID labels affixed
Two 120-mL plastic specimen bottles§ with caps and 2 specimen bottle ID labels
Seven whirl-pack plastic specimen bags§ and 7 specimen bag ID labels
Three 20-cc disposable plastic syringes¶
Three 18Gx1½"-needles¶ and 1 16Gx4"-needle¶
Eight ziplock bags and 1 marking pen
One large black plastic bag with 3 sheets of liquid absorbent material
Two reusable refrigerant packs, 1 security seal, 1 yellow plastic tape roll, and necessary tags
One preprinted return address label for the CAMI laboratory

*Each tube is sterile and contains 20 mg of potassium oxalate and 100 mg of sodium fluoride.
†Each tube is sterile and contains sodium heparin.
‡Sterile, but without additives.
§Without additives.
¶Sterile.

Table II. Types, Amounts, and Analytical Suitabilities of Specimens* Required by CAMI For Toxicological Evaluation

Specimen Type†	Optimal Amount‡	Analysis Type Suitability
Blood	40 mL	Green-top tube blood for carboxyhemoglobin (COHb), blood cyanide (CN^-), hemoglobin A_{1c} (HbA_{1c}), and drug analyses
		Gray-top tube blood for ethanol and drug analyses
Urine	100 mL	Ethanol, drug, and glucose analyses
Vitreous Humor	2 mL	Ethanol and glucose analyses
Spinal Fluid	Available Amount	Ethanol and, perhaps, drug analyses
Bile	10 mL	Abused drug§ radioimmunoassay (RIA) screening, when urine is not available
Gastric Contents¶	100 g	Need-based drug analysis
Liver	500 g	Ethanol and drug analyses
Muscle	300 g	Ethanol and drug analyses
Spleen	150 g	Ethanol and drug analyses
Lung	100 g	Ethanol and drug analyses
Kidney	100 g	Ethanol and drug analyses
Brain	100 g	Ethanol and drug analyses
Heart	50 g	Ethanol and drug analyses

*The given types and amounts of specimens are for the ideal situation wherein multiple specimen types are available in enough amounts. It is understood that this requirement may not always be met since it could be possible that some sample types are available in optimal or adequate amounts, while others may not be available at all. In such situations, analyses are performed on selected sample types. The sample selection is based on the intrinsic nature of the analytical method, analysis (qualitative, confirmatory, and/or quantitative), and analyte. Therefore, all available samples should be submitted.

†Drugs/substances (tablets, caplets, capsules, powder, and/or liquid) found at the accident scene are also occasionally submitted. Analyses of drugs/substances may be helpful in the investigation of accidents, particularly when they are also found in the submitted biological samples.

‡An adequate amount considered is half of the optimal amount. Any specimen submitted in the amount less than the adequate amount is considered to be inadequate.

§Amphetamine, methamphetamine, phencyclidine, cocaine, opiates, cannabinoids, barbiturates, and benzodiazepines.

¶All gastric contents should be submitted. In those fatalities (cases) wherein the submission of all gastric contents could not be possible, any available amount of gastric contents should be submitted. By knowing the total amount of the gastric contents, the amount of the drug/substance present in the contents can be determined.

ticular case utilized during the analytical process are automatically tracked and recorded on the batch-by-batch basis using the internal specimen-chain-of-custody form of the case.

Specimen Selection and Analytical Methodology

Green-top tube blood is preferred for the analysis of COHb, CN^-, and HbA_{1c}. If this blood is not available in sufficient amount, then gray-top tube blood is used. HbA_{1c} analysis in blood is conducted when glucose values are > 100 mg/dL in urine and/or > 125 mg/dL in vitreous fluid or upon the request of the accident investigator. COHb, CN^-, and HbA_{1c} determinations in blood are made by spectrophotometric, colorimetric, and latex immunoagglutination inhibition methodologies, respectively. Glucose levels in urine and vitreous fluid are measured enzymatically.

Alcohol/volatile analysis is performed by headspace gas chromatography (GC). The order of preference of specimens for the alcohol/volatile analysis is vitreous fluid, urine, gray-top tube blood, brain, muscle, and other tissues. If the ethanol concentration in liquid samples—for example, vitreous fluid, urine, and blood—is ≥20 mg/dL by the GC method, then those liquid sample aliquots of the batch are also analyzed by the radiative energy attenuation method to reconfirm the presence of ethanol in the liquid samples.

Attempts are always made to save some blood for confirmatory/quantitative drug analyses. Urine is preferentially used for the screening of prescription and over-the-counter drugs by high performance liquid chromatography (HPLC) and by gas chromatography/mass spectrometry (GC/MS) and of abused drugs—amphetamine, methamphetamine, phencyclidine, cocaine, opiates, cannabinoids, barbiturates, and benzodiazepines—by fluorescence polarization immunoassay (FPIA). Certain additional drugs—for example, acetaminophen, salicylate, quinidine, theophylline, phenytoin, and propoxyphene—are also screened in urine by FPIA.

In the absence of urine, other specimens are screened for abused drugs by radioimmunoassay (RIA). The order of specimen preference for the RIA-based abused-drug screening is bile, blood, liver, kidney, and other tissues. Bile is not used for the screening of prescription and over-the-counter drugs by HPLC and GC/MS; instead, blood or other tissues are used. In those cases wherein bile is not available, blood or any other tissue is used for the RIA-based abused-drug screening, and the same sample type used for RIA is preferentially used for the HPLC and GC/MS-based drug screening, as well. In other words, with the exception of bile, it is preferred to use the same specimen type for the RIA-based abused-drug screening and for the HPLC and GC/MS-based drug screening. Blood samples are also screened for acetaminophen, salicylate, quinidine, theophylline, and phenytoin by FPIA.

As mentioned earlier, confirmatory/quantitative batches contain aliquots of the sample type, which was determined as positive during the initial analysis/screening, and of at least one additional sample type of the case. Toxicologically preferred samples for these types of batches are blood and urine. Depending upon the type of analyte, these batches are analyzed by GC/MS, liquid chromatography/mass spectrometry, or any other specific technique. Confirmatory/quantitative batches for COHb, CN^-, HbA_{1c}, and glucose are generally prepared with new aliquots of the applicable sample type of the case and appropriately reanalyzed by using the methods mentioned previously in the text. Recently, a new GC method has been adopted in the laboratory for the COHb confirmatory/quantitative batch to analyze carbon monoxide in blood utilizing a thermal conductivity detector.

Reporting

After the successful analyses and reviews of all the batches, an analytical report for the case is generated. Subsequently, the entire case folder, along with the report, is reviewed for the complete case. This final review process entails accessioning, scientific, and QA/QC level reviews. After the final review, the report is signed, reviewed once more at the clerical level, and then disseminated to the appropriate authorities and agencies. A general description of the processing of aviation forensic toxicology specimens is outlined in Figure 1.

RESULTS

Biosamples from aviation accident pilot fatalities are submitted to CAMI by the NTSB for toxicological analysis. Such submissions are coordinated through the FAA's Office of Accident Investigation and the local coroner/medical examiner offices. Samples are primarily shipped in TOX-BOX evidence containers. As is outlined in Figure 1, the toxicological evaluation process involves initial analyses/screenings and confirmatory/quantitative analyses. After carefully reviewing analytical findings, toxicology reports are sent to the NTSB, FAA's Office of Accident Investigation, and other authorized agencies, such as coroners, medical examiners, and law enforcement agencies. Case-related records and analytical data are maintained

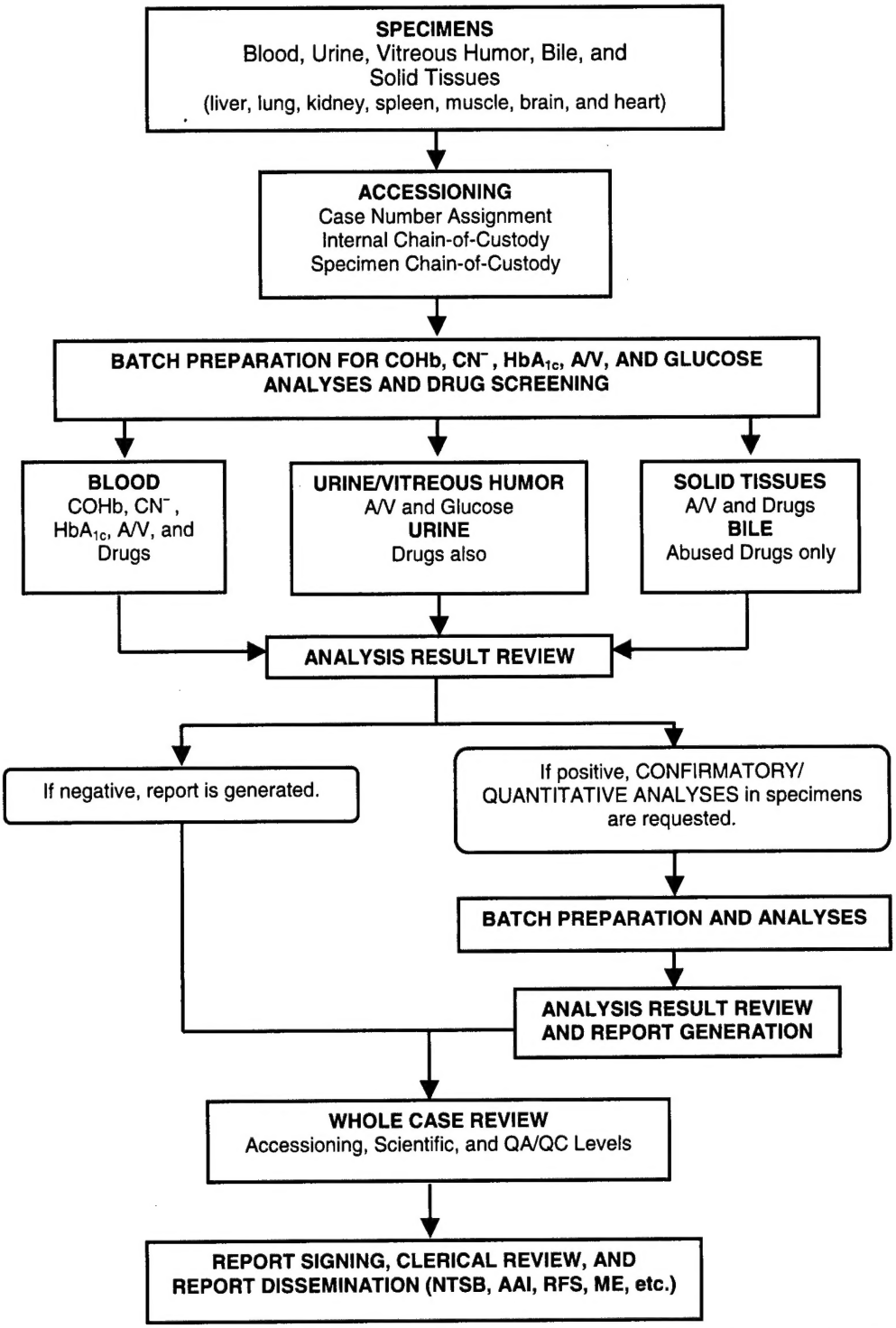

Figure 1. A flowchart for a typical toxicological processing of postmortem biological samples. Details are given in the Materials and Methods section. Carboxyhemoglobin (COHb), blood cyanide (CN^-), alcohol/volatile (A/V), glucose, and hemoglobin A_{1c} (HbA_{1c}) analysis and drug screening batches are prepared and submitted for analysis. Batches are generally comprised of samples from more than one case. Each batch contains portions (aliquots) of case specimens and quality-assurance/quality-control (QA/QC) blind samples. Combustion gases carbon monoxide, as COHb, and hydrogen cyanide, as blood CN^-, are measured since these gases are considered the 2 primary toxic gases that are produced during a fire. Drugs include prescription, over-the-counter, and abused drugs. Amphetamine, methamphetamine, phencyclidine, cocaine, opiates, cannabinoids, barbiturates, and benzodiazepines are considered as abused drugs. Abbreviations: AAI, FAA's Office of Accident Investigation; A/V, Alcohol/Volatile; CN^-, Cyanide; COHb, Carboxyhemoglobin; HbA_{1c}, Hemoglobin A_{1c}; ME, Medical Examiner; NTSB, National Transportation Safety Board; QA/QC, Quality-Assurance/Quality-Control; RFS, Regional Flight Surgeon.

at CAMI. Unless there is pending litigation, samples of negative and positive cases are properly destroyed after 2 and 5 years, respectively, from the date of their receipt at CAMI.

As is evident from Figure 2, CAMI received samples from the majority of the aviation accidents that occurred during 1990-2000. Over this period, a total of 5,051 aviation accidents were reported by the NTSB. Out of these 5,051 accidents, 237 were foreign aviation accidents. The 11-year averages were 459 (408-512) for NTSB-reported all fatal accidents, 438 (365-509) for NTSB-reported United States fatal accidents, and 354 (324-400) for NTSB-reported United States fatal accidents from which CAMI received samples (CAMI accidents). For the 1990-2000 period, samples were received from the pilot fatalities (CAMI cases) of approximately 80% of the total United States aviation accidents (4,814) reported by the NTSB. The percentage of the 1990 NTSB-reported United States fatal accidents from which biological samples were submitted to CAMI was 73%. This percentage slowly increased in the ensuing years and reached 92% for the year 2000. The gap narrowing between the number of accidents reported by the NTSB (NTSB accidents) and the number of accidents from which postmortem pilot samples were submitted to CAMI (CAMI accidents) is exhibited in Figure 2 (also, see the figure insert). The 11-year average of CAMI cases was 473 (396-585), with the highest number of 585 cases in 1991. Monthly averages for the 1990-2000 aviation accidents from which CAMI received samples and associated CAMI cases indicated the highest number of accidents and cases during June-September (Figure 3).

Optimal, adequate, and inadequate amounts of various sample types are mentioned in Table II. Although it is not always possible to obtain all samples in optimal amounts, more than half of the cases

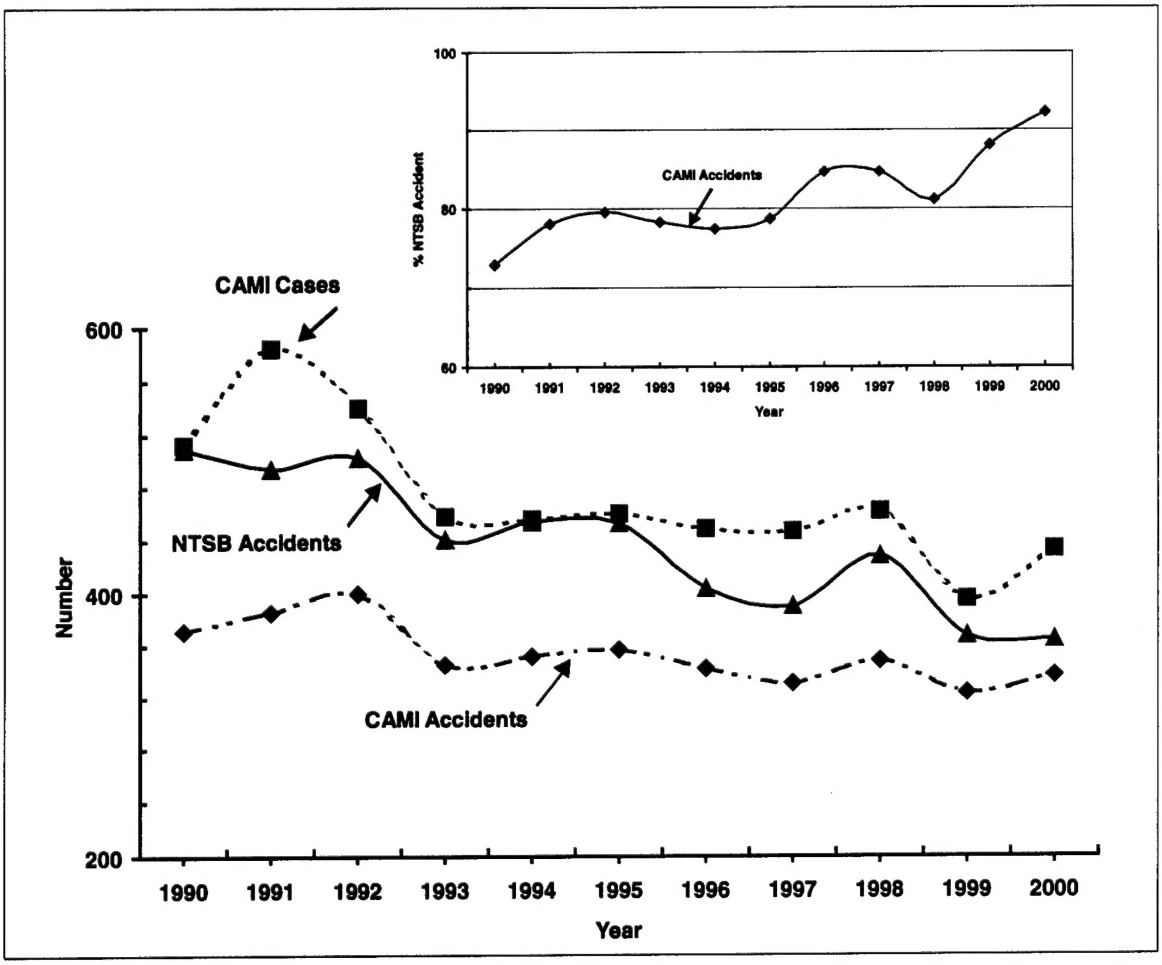

Figure 2. Numbers of the NTSB-reported 1990-2000 United States fatal aviation accidents (NTSB accidents) *versus* numbers of those aviation accidents (CAMI accidents) from which biological samples of the fatalities (CAMI cases) were received at CAMI. Insert: Percentage of the total 1990-2000 United States fatal aviation accidents reported by the NTSB (NTSB accidents) from which samples were received at CAMI (CAMI accidents).

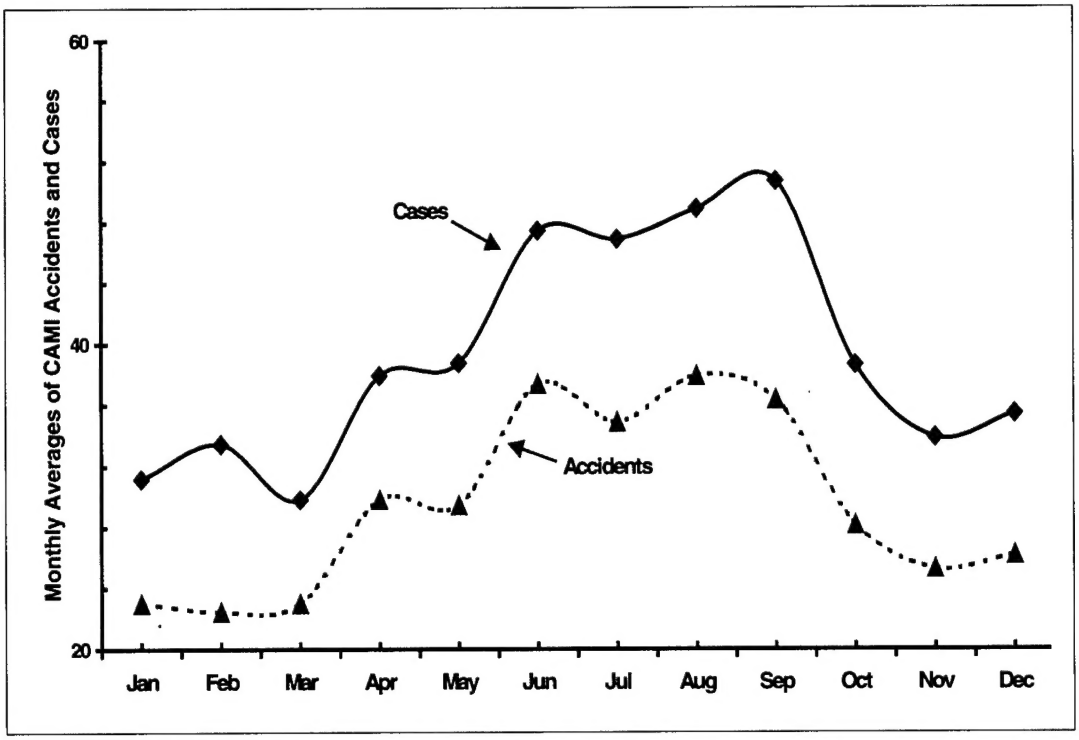

Figure 3. Monthly averages of the United States aviation accidents and associated cases from which postmortem samples were received at CAMI. These accidents occurred during the 11-year period of 1990-2000.

received at CAMI generally had multiple samples in sufficient amounts to allow toxicological analyses (Figures 4 and 5). For example, out of 1,891 cases processed for the 1996-2000 accidents, 1,211 cases had adequate amounts of blood, urine, and/or vitreous humor; 324 cases had inadequate amounts of blood and urine; 356 cases had no blood or urine. Muscle, liver, lung, and/or kidney samples were submitted in 90% of the cases, while cerebrospinal fluid was submitted in only 8% of the cases (Figure 5). The toxicologically preferred samples, blood and urine, were available in 78% and 56% of the 1,891 cases, respectively. Out of 51 cases containing only 1 sample type, 46 cases had muscle, 3 cases had vitreous humor, brain, or spleen, and the remaining 2 cases had unidentified body tissues. Optimal amounts of heart, brain, kidney, lung, blood, and vitreous fluid samples were submitted in 64, 56, 53, 49, 39, and 37% of the 1,891 cases, respectively (Figure 5). Considering CAMI's sample requirements (Table II), urine samples were submitted in optimal amounts in only 2% of the 1,891 cases and in adequate amounts in 16% of the 1,891 cases. After combining "adequate" amounts with "optimal" amounts, it was determined that heart, brain, kidney, lung, and blood samples were submitted in 60-80% of the 1,891 cases.

DISCUSSION

One of the important components of aviation accident investigations is forensic toxicology of postmortem biological samples collected from pilot fatalities. In the United States between 1990 and 2000, biosamples from approximately 80% of all civilian aviation accidents were submitted to CAMI. Failure to receive the samples from the remaining accidents could be attributed to (i) unrecoverable pilot remains from accident sites, (ii) religious belief of the deceased pilot's family in not performing autopsy and postmortem analysis, and (iii) accident investigator-in-charge's decision not to seek forensic toxicology or not to send samples to CAMI. The increase in the number of CAMI accidents seen for the 2000 accidents in relation to the 1990 accidents is probably the result of concerted professional interactions of CAMI accessioning staff with the NTSB contact person(s) and FAA's Regional Flight Surgeons. The enhanced interaction has been achieved by providing these groups weekly, by E-mail, a list of those aviation accidents from which samples were not received at CAMI. These messages led the respective E-mail receivers to determine the reasons for not sending the samples and, if necessary, to expedite the sending of

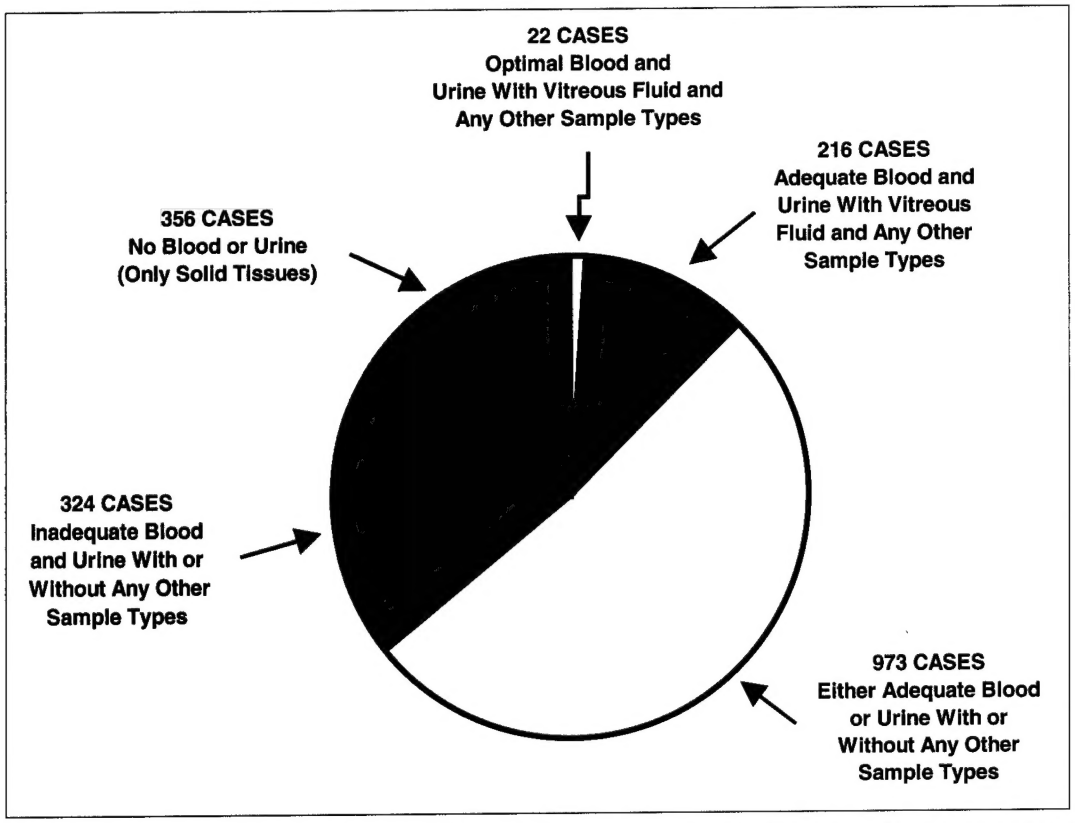

Figure 4. Specimen type and amount-based categorizations of postmortem toxicology cases received at CAMI from the United States fatal aviation accidents that occurred during 1996-2000. A total of 1,891 cases (fatalities) were received.

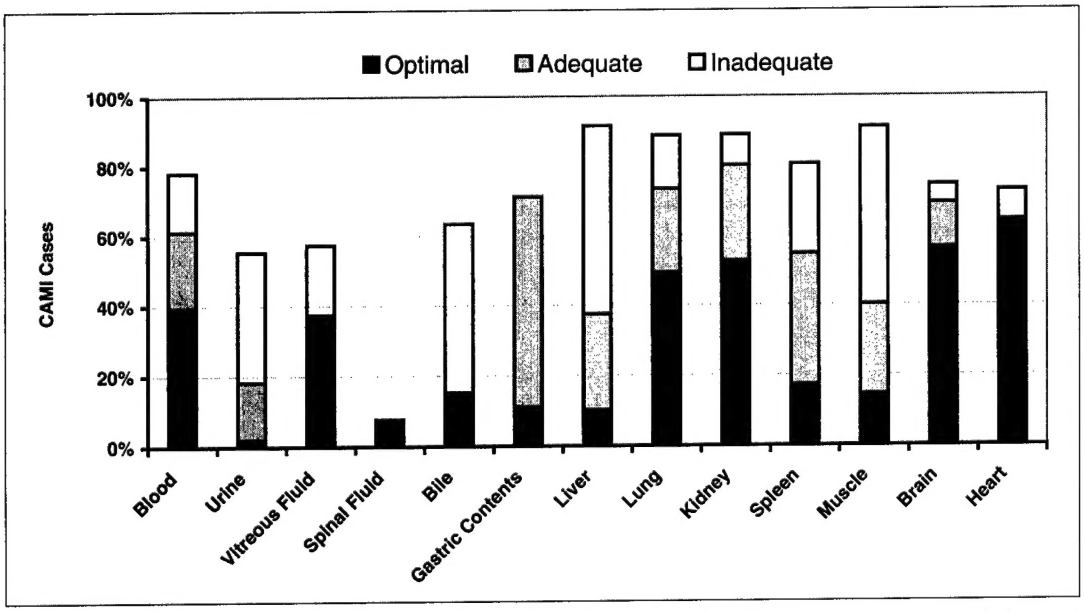

Figure 5. Optimal, adequate, and inadequate amounts of various postmortem biological specimens received at CAMI from the 1,891 cases (fatalities) of the United States fatal aviation accidents that occurred during 1996-2000.

samples. This increased contact of CAMI was initiated in late 1998, and its positive effect has become clearly evident in 2000.

The higher number of "CAMI cases" in relation to the respective number of "CAMI accidents" was obviously attributed to more than 1 occupant in some of those accident aircraft. The highest number of CAMI cases as seen for the 1991 accidents was, at least, partly attributed to the samples from the fatalities of the 01 February 1991 Los Angeles accident involving 2 aircraft (15,17) in which there was a fire. Therefore, in addition to the samples from pilots/copilots, samples from other occupants were also submitted for COHb and CN^- analyses. In that 2-airplane runway collision, samples from a total of 33 fatalities were submitted. The observed pattern of the increased accidents and associated cases during the summer months (June-September) could be ascribed to the higher aviation activities during these months.

Each aviation accident is different, and so is the postmortem toxicology of the accident-associated fatalities (cases). Obtaining multiple sample types in sufficient amounts is not always possible since it is governed by the nature of the accident. Differences from case to case are attributed to the availability of sample types, amounts of samples submitted for analysis, numbers and concentrations of analytes present in those samples, and chemical nature of the analytes. Instead of obtaining all the desired sample types in optimal amounts for an accident, only certain sample types are sometimes available in optimal or adequate amounts, while others in inadequate amounts or not available altogether. In those scenarios, analyses are performed on selected sample types, and their selection is based on the intrinsic nature of the analytical method, analysis type (qualitative, confirmatory, and/or quantitative), and analyte.

Pharmacological agents in pilot fatalities are generally present in the subtherapeutic to therapeutic concentration range (7,8,16). This observation is consistent with the very fundamental difference between general forensic toxicology and aviation forensic toxicology (9). The former field—wherein toxic-to-lethal levels of drugs are often involved—primarily deals with the cause and manner of poisoning/death, whereas the latter field with the cause of accident. In this way, "postmortem aviation forensic toxicology" is basically a human-performance associated postmortem forensic toxicology endeavor (13). Having multiple sample types in sufficient amounts is essential for postmortem aviation forensic toxicology because this facilitates the analyte and/or analytical method dependent selection of sample types. For example, blood from the green-top (heparin) tube is more suitable for COHb, CN^-, and HbA_{1c} analyses since it is more homogeneous than the blood from the gray-top (potassium oxalate and sodium fluoride) tube. Blood from the gay-top tube is appropriate for ethanol analysis because of the antibacterial properties of sodium fluoride. The presence of sodium fluoride prevents the bacteria-mediated *in vitro* production of ethanol after blood collection. Ethanol analysis in vitreous fluid, urine, and brain is essential for establishing the postmortem production of ethanol (6,14,18). Since vitreous fluid and brain are anatomically preserved, the potential for their exposure to bacteria is usually less. Also, the bacterial growth is relatively less in urine because of its physiological nature. Thus, the potential for putrefaction and subsequent postmortem ethanol production is less in vitreous fluid, urine, and brain than in other biofluids or tissues, including blood. Glucose analysis can be conducted more accurately in vitreous fluid and urine than in postmortem blood. However, HbA_{1c} concentration determination in blood is crucial in establishing the existence of hyperglycemia and overall glycemic control (5,20).

Depending upon distribution characteristics, certain drugs may not be present in appreciable amounts in some body compartments, whereas they may be present in considerable amounts in other compartments (3,4,9,12,19). Conjugates of drugs and their metabolites are generally found in appreciable amounts in bile. Therefore, this matrix is suitable for detecting those drugs, which may not be present in appreciable amounts in other compartments. Bile can effectively be used for detecting drugs, including abused drugs, by RIA. However, this sample type may not be suitable for the screening of prescription and over-the-counter drugs by HPLC and GC/MS because this matrix produces extraneous peaks on the chromatograms, thereby interfering with the analysis. Urine is an appropriate matrix for finding drug metabolites, as well as basic drugs. In blood, drug metabolites usually do not accumulate much and basic drugs are generally present in low concentrations. Compared with basic drugs, neutral and acidic drugs are commonly present in higher concentrations in blood. Demonstrating the presence of drugs and/or their metabolites in multiple sample types provides convincing scientific evidence

for the exposure of fatal accident victims to those drugs. Such findings are important when metabolites are not available as drugs, suggesting that the metabolites were biotransformed *in vivo* from the parent drug.

The present study documents that sufficient amounts and types of biological samples are being submitted from the majority of the aviation accidents to ensure thorough toxicological evaluation. As a standard practice at CAMI, the presence of analytes must generally be established in at least 2 different sample types by using 2 different analytical techniques for reporting a particular case as "positive," provided sufficient amounts of samples are available. If there is only 1 sample type in a particular presumptive positive case, 2 different portions of the sample are usually analyzed prior to reporting as a positive case. Similarly, a different aliquot of samples is reanalyzed for specimen specific analysis, such as COHb and CN^- analyses in blood. Initial analysis/screening and subsequent confirmatory/quantitative analysis could be considered 2 types of analyses.

During the whole toxicological process from the case specimen receiving in the accessioning area to the analytical result reporting and the data/record keeping, an effective QA/QC program is enforced by multilevel reviews. The CAMI laboratory participates in the forensic urine drug and whole blood alcohol/volatile proficiency-testing surveys and in the accreditation/certification program of the College of American Pathologists (Chicago, IL) to ensure the highest level of integrity. Additionally, the laboratory participates in the FAA's postmortem forensic toxicology proficiency-testing program (11), which is one of the few proficiency-testing programs recognized by the American Board of Forensic Toxicology Laboratory Accreditation Program (1).

In conclusion, CAMI receives postmortem pilot specimens from the majority of the United States fatal civil aviation accidents. Such submitted samples are generally of multiple types in sufficient amounts. Toxicological evaluation is conducted by using selective and sensitive analytical procedures in multiple sample types. During the entire process, the highest level of integrity and quality is maintained.

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